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Abstract

There is a need for formalised diagrams that both summarise current biological pathway knowledge and support modelling approaches that explain and predict their behaviour. Here we present a new, freely-available modelling framework that includes: a biologist-friendly pathway modelling language (mEPN); a simple but sophisticated method to support model parameterisation using accessible biological information, a stochastic flow algorithm that simulates the dynamics of pathway activity, and a 3D visualization engine that aids understanding of the complexities of a system's dynamics. We present example pathway models that illustrate of the power of approach to depict a diverse range of systems.

How to draw diagrams of biological pathways?

Biologists the world over love a good diagram. The old adage of a picture being worth a thousand words holds true. Diagrams continue to form the bedrock of our efforts to communicate ideas about the complexity of biological systems and pathways. They act as pictorial reviews of what is known about the components of a

given system and how they interact. However, there is a fundamental problem with most biological pathway diagrams: they are produced on an *ad hoc* basis with no rules governing their composition or content. As a result they convey information only in the narrow context of the accompanying legend, text or talk, and as a collective resource are of limited use as they cannot be directly compared or integrated.

If pathway modelling is to be more widely adopted by biologists, and the results of their efforts useable by others, a new approach is required. Here we describe a modelling framework that consists of a formalised graphical language that supports the depiction of complex biological interactions and molecules allowing the creation of detailed models of events based on known information. Once parameterised, these models can then used directly for experiments that seek to simulate their activity under a variety of conditions. The approach utilises flexible and simple-to-use tools that make diagrams easy to draw and edit as new knowledge arises, and to model their behaviour. Most importantly the resulting pathway diagrams are useful; in the communication of ideas, in hypothesis testing, in enhancing the understanding of the dynamic characteristics of the system modelled, and thereby in helping to drive forward biological research objectives.

The post-genomic era has led to the generation of a massive amount of data on the interactions between molecules across cell types and disease processes. Pathguide.org [1] lists and categorises many of the available resources proving access to this information and currently lists 547 websites and databases (as of May 2016). Many are databases of collated biological interaction data, experimentally determined or inferred, some well-known examples including String [2], GeneMania [3], ConsensusPathDB [4], IntAct [5] and BioGrid [6]. Other initiatives such as Pathway Commons [7] and PSICQUIC [8] have sought to ensure there are common standards to exchange this information. However, these resources store and serve up interaction data primarily as pairwise interactions, where the context, nature or result of these interactions are generally not shown. In other words the data exists as a simple network of interactions not as a formalised pathway diagrams. Representing the interactions between biological components as pathways is a challenge and a number of notation schemes have been proposed [9,10,11,12,13,14,15], culminating in the establishment of the Systems

Biology Graphical Notation (SBGN) project. In 2009 the SBGN community proposed a number of standards for pathway depiction including the 'process description language' [12] based on ideas first proposed by Kitano *et al.* [9]. In process description diagrams, components of a pathway are depicted using a standard set of shapes (glyphs), and both the nature of the interactions between components and the products of those interactions must be shown explicitly. Since its original description a number of individual models have been constructed based on this approach [16,17,18,19,20,21,22], and a number of databases [23,24,25,26] now provide centralised pathway resources constructed using a SBGN-like process description notation. Other popular pathway diagram databases such as KEGG [27] and WikiPathways [28] use their own notation systems. Whilst adoption of SBGN's process description language by parts of the community undoubtedly represents a step in the right direction, many aspects of the SBGN scheme are limiting; the depiction of many common concepts in biology are not supported, the computer science-based language used to describe it can be alienating to biologists, and tools that support SBGN pathway construction are not always easy to use or have limited functionality.

A biologist friendly approach to pathway modelling

A number of years ago we started to explore the problem of how does one draw a 'good' diagram of a biological pathway. In order to ensure the usability and widest appeal of our approach, we have strived to ensure that the diagrams (graphical models) produced were able to depict events as described in the literature in as much detail as possible, whilst maintaining their readability. We also wanted the approach to be understood and useable by relatively junior biologists, as in many cases they were the ones constructing models. To these ends we developed the modified Edinburgh Pathway Notation (mEPN) scheme (for an up to date version see Fig 1). In common with SBGN the mEPN scheme is also based on the principles of the process diagram (see Box 1 for a comparison of the mEPN scheme and to SBGN). The mEPN scheme is designed to be clear, concise and embraces the use of colour to enhance the readability of diagrams. The excellent yEd (www.yWorks.com) software is the primary pathway drawing tool providing a platform that is robust, flexible and free to use. It has a range of features that allow a user to link parts of the model to external resources or

other supporting data (e.g., pdf files of informative papers and websites), and provides an easy to use platform for diagram visualisation and editing. An extended description of construction of mEPN models is provided in S1 Text and a protocols paper where we have sought to provide a detailed guide to any would-be pathway modeller is available on bioRxiv preprint server (http://dx.doi.org/10.1101/047043) .

Fig 1: The modified Edinburgh Pathway Notation (mEPN) scheme, version 2.0. Nodes are classed as being either a 'place' or 'transition' and are connected by 'edges'. The scheme consists of 12 different entity nodes (top left panel) representing different kinds of molecular species. Also included are a Boolean logic 'OR' operator node and a spacer node that may be required to maintain bipartite structure. Entity nodes are annotated to describe the identity and state of the entity e.g., protein symbol and its modifications (bottom left panel). Also included in the scheme are 45 different process nodes (top central panel) the majority representing different interactions that can take place between place pathway entity nodes. Also included in this class of nodes are a Boolean logic 'AND' operator node, a sink node, two token input nodes and a spacer node. There are six possible connecting edges (bottom central panel) that represent the nature of the interaction between a place and a transition, two of which are inhibitor edges and one of which is a non-directional edge that represents a non-covalent interaction. Components are represented in the context of their subcellular location represented by 'container' nodes of specific colours (right panel). In all cases colour is not essential to defining node type but is designed to aid visual distinction between them.

Box 1: mEPN versus SBGN

SBGN is perhaps the most widely employed graphical notation scheme in use today for pathway modelling. The objectives of SBGN are aligned with our own, and the mEPN scheme has borrowed concepts from it. Indeed, models constructed using the mEPN scheme may be exported from within BioLayout *Express*^{3D} as a SBGN-ML file, from whence they may be viewed and edited within tools that support SBGN e.g., Vanted[29] (Fig 3). In our experience however the SBGN scheme is overly complicated for an audience of biologists, is limiting in terms of its ability to depict certain processes and we believe the mEPN scheme offers certain

advantages. However, where possible we have attempted to maintain consistency with the SBGN standard but there are a number of areas where we have chosen to deviate from it:

1. **SBGN uses a number of non-geometric shapes to depict a number of pathway entity types (or EntityPoolNodes as they are referred to in SBGN). These shapes are not supported by yEd or indeed other generic network editors and are not used in the mEPN scheme.**
2. **Protein complexes are represented in SBGN using either a 'stack of proteins' glyph in the case of homo-multimers or as a series of individual protein subunit glyphs within container node in the SBGN scheme. In mEPN all such entities are represented as a single rounded rectangle glyph where the number and name of constituent entity nodes are defined by the node's label; we see no advantage graphically or otherwise in the rendering complexes by the means proposed by SBGN. In drawing larger complexes we have also often found it useful to show individual proteins as individual entities joined by lines with no arrows (the so called 'non-covalent interaction' edge) so that the structure and relative position of individual subunits might be better communicated graphically (see Fig 2B: a model of the nuclear pore). However, such models cannot currently be used in the computation modelling environment as they do not conform to requirements for a bipartite graph structure of computational models.**
3. **SBGN has specific glyphs for association (binding) and dissociation but almost all other processes are represented by a square box. This gives no information on the process they represent and is an important omission. In mEPN we currently list 40 types of processes that one might wish to define when constructing a pathway, including all of those associated with the BioPAX level 3 pathway exchange language[30]. Whilst in the computation modelling environment of SPN all transition nodes function the same, it is important to differentiate between what are often quite different processes in order to aid understanding and readability of the models. We have added the black rectangle and diamond transitions for modelling purposes.**
4. **We have simplified the list of edges representing interactions between entity nodes down to interaction, catalysis, inhibition and the special use case action potential, and do not recognise many of the options available listed by SBGN i.e., consumption, production, reversible modulation, necessary stimulation,**

logic and equivalence arcs, as we have found them difficult to explain to biologists and not necessary to the depiction of pathways.

5. **Finally, and perhaps the most important issue for computational modelling purposes is that in the current version of mEPN we define all nodes as being either places or transitions, a division that makes biological sense in terms of what they represent, and conforms to the requirement of Petri net models.**

An example of a simple model of the signalling pathway of Interferon-β (IFNB1) represented in the mEPN scheme, BioLayout *Express*^{3D}, an SBGN diagram or a Petri net can is shown in Fig 3 for comparison.

At its most basic level a pathway diagram constructed using the mEPN scheme is a simple network composed of nodes and edges. Nodes fall into one of two categories, representing either pathway entities or processes. Entity nodes generally represent biomolecules e.g., RNAs, proteins, complexes, biochemicals, and each class of entity is represented by a glyph of a particular shape. Process nodes represent an event occurring to or between entities e.g., translocation, binding, dissociation, phosphorylation, and are generally represented by a circle with a 2-3 letter label distinguishing between different types of processes they represent. The lines that join nodes, called edges, define the direction of an interaction and in some cases the nature of it e.g., catalysis, action potential, inhibition, or non-covalent interaction. A model of the well-known pathways of the glycolysis and the TCA cycle constructed using mEPN is shown in Fig 2A, and an example of how a large complex such in the nuclear pore can be shown to be made up of multiple connected subunits using the non-covalent edge is shown in Fig 2B (these models are also available to download and edit in S1 GraphML).

Fig 2: Examples of pathways and complexes represented using mEPN. (A) A model of the human TCA cycle and glycolysis pathways drawn using the mEPN scheme. The insert shows a small section of the pathway demonstrating the different shapes and colours used to depict different classes of molecule and how different types of processes can be distinguished using differently labelled process nodes. This is a purely graphical model but with parameterization could be used to run simulations of this pathway. (B) Depiction of the nuclear pore complex as an example of how the mEPN scheme can be used to represent large

structures composed of multiple subunits. In all models constructed by us, standard nomenclature is used to define entities e.g., HGNC approved names for human proteins and genes, to remove ambiguity of what is being represented.

S1 GraphML: GraphML files of the models shown in Fig 2.

Beyond the picture

A hallmark of many modern biological investigations are genome-scale analyses which continue to produce huge amounts of data. These inevitably implicate hundreds or thousands of biological entities as being of interest due to their change in abundance in a given experimental or clinical paradigm. However, interpretation of the meaning of such observations represents one of the greatest challenges in biology today. Gene Ontology (GO) enrichment analyses, may indicate that a set of genes or proteins 'hits' are implicated in a given pathway or process, but what role they play or how they function together is not defined. Similarly, and as mentioned above, protein-protein interaction databases may be used to find out if there is any evidence that a set of proteins share a functional association, but the information returned is generally without context. An alternative approach is to overlay results on to pathway diagrams thereby indicating which of those represented have been regulated. The researcher can then understand not only which pathways have undergone regulation but which specific elements have been altered, allowing them to hypothesise how a given perturbation might affect a pathway's activity or downstream events. This method is very useful but limited by the quality and scope of the reference pathway diagram. There are a number of tools that currently support data visualisation in the context of networks and pathways including CellDesigner [31], NaviCell [32], KEGG Mapper [27], ReactomeFiViz [33], iPath [34] and Medusa [35]. As knowledge of biological interactions

177 grows, there is therefore a need to construct better models to support these types of analysis and we present
178 here our to constructing such models.
179
180 This brings us to perhaps the most challenging aspect of pathway modelling, that of simulating their activity.
181 To truly comprehend a pathway one needs not only the pictorial representation or 'connectivity map' but also
182 to understand the dynamics of the system under different conditions. Computational and mathematical
183 modelling studies aim to simulate biological systems to explain a pathway's observed activity. When this is
184 achieved, models can be used to explain or predict a system's response to perturbations e.g., a drug treatment,
185 gene knockout etc. or used to predict missing components. Over the years numerous computational models
186 have been published for a diverse assortment of biological systems (see the BioModels database for examples
187 [36]). Researchers have used a range of approaches to simulate system dynamics including ordinary and partial
188 differential equations, qualitative differential equations, stochastic equations, directed graphs, Bayesian and
189 Boolean networks, and rule-based formalisms [37,38] and the Systems Biology Markup Language (SBML) has
190 been developed as an open interchange format for such models. Most equation-based models, are described
191 primarily by the maths and graphical representation of the events being modelled is generally an afterthought.
192 Indeed, whilst mathematical modelling of biological pathways was one of the founding activities of systems
193 biology, currently pathway depiction and pathway modelling are generally considered to be separate
194 disciplines. The aim of one is draw an accurate representation of known events, the other to understand how
195 a pathway might operate as a dynamic system, and the two activities are generally not compatible. However,
196 it is the requirement for experimentally derived rate constants to feed into many of these models and the
197 significant computational power required to solve a series of equations that generally limits equation-based
198 approaches to modelling relatively small and well characterised systems [39]. These factors have limited the

routine use of modelling by biologists who are generally are not comfortable with mathematical modelling techniques employed.

Modelling pathway dynamics using activity flow simulations

Process diagrams share much in common with Petri nets, a stochastic modelling approach that has been used to model many different types of systems, with a growing literature describing their use in modelling biological pathways [40,41,42]. Petri nets are a rule-based approach founded on the concept of modelling the flow of tokens through a system, where tokens represent the activity (or amount) of a given component. Petri nets are networks made up of two types of node: places and transitions (usually represented by circles or black rectangles, respectively). A strict requirement of Petri nets is that they are constructed as bipartite graphs i.e., they have a recurrent structure of place-transition-place (or in the case of process diagrams, entity–transition–entity). The signalling Petri Net (SPN) algorithm we have employed to dynamically model flow through mEPN diagrams was originally described by Ruths *et al.* [39], which after refinement was incorporated into the network visualisation and analysis software BioLayout *Express*^{3D} (www.biolayout.org). As part of this development we enabled the import and visualisation of mEPN models and thereby the simulation of activity flow through such models. In order to achieve this end we modified the mEPN scheme to define all nodes as representing either places (which includes all pathway entities), or transitions (which includes all nodes representing processes). An extended description of the SPN modelling system is provided in S1 Text and in the accompanying protocol paper (http://dx.doi.org/10.1101/047043).

S1 Text: Methods and an extended description of the SPN modelling system

Models need to be parameterised prior to running a simulation i.e., the initial conditions for the experiment need to be set. With this modelling platform parameterization is in principle relatively simple to perform and based on information that may be available to a biologist. The primary parameterization is the structure of a model itself; this is the major determinant of the where tokens can flow to and from, and how long it takes

for them to move from place to another. Parameterization is also carried out through the placement of tokens on entities at the starting points of a network i.e., on places that have no parents. An entity without tokens, or where token number is set to zero, can be considered not to exist (e.g., a knockout or not expressed). The more tokens placed on an entity node prior to running the model the more of that entity (or greater its activity) at the beginning of a simulation. The assignment of tokens to an entity node can be arbitrary or inferred from experimental evidence, such as gene expression or proteomics data, where available.

Models constructed using the mEPN scheme, parameterised and then saved in yEd as GraphML files can be loaded into the tool BioLayout *Express*^{3D}. BioLayout has been designed to recognise the different glyphs used in the mEPN scheme and translates all 2D mEPN glyphs into their 3D equivalents e.g., circles become spheres, rounded rectangles become rounded cuboids (Figs 3A&B). The 3D palette of shapes being far larger, allows greater flexibility to distinguish between certain entity types. BioLayout can also export a SBGN-ML version of a mEPN model such that it can be loaded into an SGBN compliant platform such as SBGN-ED [43] as used here (Fig 3C). Shown in Fig 3D is an illustration of how the concepts of the mEPN language map onto the bipartite graph structure of a Petri net.

Fig 3: A simple pathway represented in four different ways. This figure represents a simplified model of the interferon-β signaling pathway. (A) Shows this pathway represented using the mEPN scheme and parameterized ready to run a simulation, (B) when imported into BioLayout *Express*^{3D} and visualised using the mEPN 3D notation scheme, (C) as exported from BioLayout as an SBGN-ML file and loaded in the Vanted software, and (D) the same set of interactions represented as a Petri net, illustrating the similarity to mEPN diagrams.

When a mEPN model is loaded, BioLayout requests whether a simulation is required. On answering yes a dialogue is presented (S1 Fig) by which conditions for the SPN simulation can be set i.e., mode of stochasticity, number of runs and time blocks etc. A simulation is made up of a series of 'time blocks' in which each transition

in the model is 'fired' exactly once in a random order and tokens are moved from the place(s) upstream of the transition to the downstream place(s). In the default mode the number of tokens moved is essentially random i.e., between the 0 and the maximum number of tokens available. A series of time blocks is referred to as a 'run' and a simulation may involve one or many runs, each run beginning at the same initial token marking. The result of a simulation for a given pathway entity node (place) is the average accumulation of tokens across individual runs and as such is associated with a measure of variance. The more runs performed, the more reproducible results will be across separate simulations. It should be noted that even when running simulations on large models, over many time blocks, composed of hundreds of runs, computational times for the SPN algorithm are normally no more than a few seconds (S1 Fig). Following a simulation run the activity of the system and individual entities within it, are represented by the flow of tokens from one entity node to another over time. One way to view results is through the animation of this token flow, a node's size and colour being used to represent token accumulation over time. This is a capability enabled within BioLayout, which has an interface that allows the user to control many aspects of this animation (S1 Fig). This functionality provides a powerful visual medium to appreciate token flow in large network diagrams and can be used to troubleshoot the innate challenges associated with model construction. Indeed, the general *modus operandi* when optimizing models, is to construct them in yED, test the flow characteristics in BioLayout and to solve issues encountered by going back to the model in yED and then retesting the model. In reality the movement between the two tools is quick and model testing and improvement can be a rapid process. See S2_Movie for a summary of the modelling process.

S1 Fig: Setting up BioLayout Express^{3D} to run and visualize SPN simulations. (A) When an SPN-ready model is loaded in BioLayout *Express*^{3D} the software automatically recognizes it and prompts users to run a SPN simulation using this window. The user defines the number of time blocks and runs, whether the variance is displayed, the form of stochasticity that the model is parameterized with and the transition type. The set-up used for generating visualizations in this article is illustrated. (B) On completion of the SPN simulation run, this window provides a time for the running of the simulation and prompts the user to repeat the

simulation, save the SPN results or open the animation dialog. (C) The animation control dialogue window allows the visualization of the SPN simulation to be defined. Size and colour of nodes as well as the appearance of token flow and the speed of the animation can be defined here.

Flow characteristics through basic pathway motifs

Linear flow

In a linear network consisting of a line of alternating places and transitions, each time a transition is fired tokens will 'flow' down the network, the more tokens added the more tokens will accumulate downstream (Fig 4A.i). With a constant input of tokens, token accumulation rises to a constant level and remains 'steady', the rate of tokens entering a place approximating those leaving; places close to the start of the line accumulating tokens faster than those further downstream (Fig 4A.ii). When tokens are added in for a fixed number of time blocks i.e., a pulsed input, a wave of flow is produced, the amplitude of the wave reducing and its wavelength increasing at places downstream of the input (Fig 4A.iii). Variation of flow over time under steady state conditions, depends on the number of runs performed and the randomness of signal propagation (Fig 4B).

Fig 4. Flow simulation through simple pathway motifs (A) A linear network with tokens added to the system on the first node (X) illustrates how differences in the placement of tokens and SPN parameters affect token output flow. Token accumulation on the blue, red and green nodes in the network has been used to illustrate the effect changing the number and/or pattern of tokens introduced. (i) Varying the initial token input (X=50, 100 or 200), (ii) the rate of token accumulation at different points downstream of a constant input (X=100), and (iii) token flow following a pulsed input of tokens (100 tokens during time blocks 11-30). (B) Token accumulation on the blue node illustrates the effect on changing the number of runs and stochasticity of the SPN algorithm (X=100). (C) Modelling multiple inputs into a place. The red node shows two places feeding into a downstream place via a transition node where flow is determined by the upstream node with the least number of tokens. Input to the green node is via separate transitions token

accumulation is additive. (D) Modelling multiple outputs from a place. In the first instance, where output from the blue node is via a single transition the yellow downstream node receives the same number of tokens as was present on blue i.e., flow is conserved. When outputs are split from the node itself, token output is distributed equally amongst downstream nodes (purple). (E) Negative feedback loops. In the first case a non-competitive inhibitor edge (i., red edge with a flat bar) will completely stop token flow through the target transition if any tokens are present on the inhibitor node. By contrast, competitive feedback edge (ii., red edge with an open diamond end), the number of tokens on the inhibitor node are subtracted from the number of tokens flowing through the target transition. An additional factor in determining flow through a negative feedback loop is the number of steps between input and feedback. The more nodes that are present between feedback receiver and inducer, the more tokens accumulate in the system resulting in a longer the wavelength of the feedback will be and the greater the amplitude of token flow, as illustrated here by the differences between 1, 10 and 50 nodes between signal input and inhibitor. All token flow plots are shown as mean (darker line) with standard error (lighter area around line).

Flow through interactions between multiple entities

Pathways are made up of a small number of basic network motifs [44]. When transitions receive numerous inputs they function as rule-based regulators of token flow. A basic rule is that when the number of tokens on input places is not equal, the number of tokens taken forward will be based on the input place with the least number of tokens (Fig 4C, red). In the case of multiple inputs into places, inputs are additive (Fig 4C, green). In instances where there are multiple outputs from a transition, the number of tokens on the upstream place will be mirrored by downstream places i.e., flow is preserved (Fig 4D, yellow). Where outputs come direct

from a place, the number of tokens on the upstream place will divided randomly amongst downstream places i.e., flow is divided (Fig 4D, purple).

Feedback control

Feedback loops are a fundamental feature of biological systems and an ability to model them is an essential property of any modelling system. Inhibitor edges have a unique function. They originate from an entity (the inhibitor) and connect with a transition node i.e., the process that is to be inhibited. This system includes two types of inhibitor edge representing non-competitive and competitive inhibition. The first operates on the basis that if any tokens are present on the inhibitor, flow through the transition is completely blocked. In the second case the number of tokens residing on the inhibitor is subtracted from the number of tokens flowing through the transition. As no tokens are lost through inhibitor edges it has become standard practice to draw inhibitor places with an associated output transition (sink). Without this flow through a negative feedback loop is completely and irrevocably stopped as tokens accumulated on the inhibitor remain there blocking further flow through the target transition. An inhibitor with a sink effectively has a half-life and depending on the configuration of the feedback loop e.g., path length between input and inhibitor and type of inhibition (amongst other factors), the system may exhibit a range of oscillatory activities (Fig 4E).

Described above are the characteristics of flow simulations through basic pathway motifs. It should be noted that this method is scalable and large pathway models consisting of hundreds or possibly thousands of nodes can be constructed and used in flow simulation experiments. We present a recently constructed model of the influenza life-cycle in macrophages as an example of such a large-scale model (Fig 5). See also S2 Text, S2 Graphml, S2 Fig and S3 GraphML for a description of this pathway and for access to the model itself.

Fig 5: A model of the influenza A life cycle. (A) Representation of a large model of the life cycle of the influenza A virus (IAV) and interactions with the host defence systems in a macrophage. The boxes highlight the modular structure of the model. Modules of the viral replication pathway are indicated with black boxes,

modules of the macrophage host defence mechanisms are indicated with pink boxes. Model constructed and visualised in yEd. (B) Visualization of token flow through the IAV/macrophage model after SPN simulation in BioLayout *Express*^{3D}. (C) Approximately 14,000 tokens accumulate on the 'virus output' node during SPN simulation of the model when the host defence systems are removed (red) compared to ~4,000 for a naïve macrophage (purple) or <10 for an interferon primed macrophage (pink). (D) Pro-apoptotic signalling reaches a peak of approximately 16,000 tokens in interferon primed macrophages, and 14,000 in naïve macrophages, but pro-apoptotic pathways are little activated in the epithelial cell model (<800 tokens). For more details on the assembly and parameterization of this pathway and to download the pathway itself, see S2 Text, S2 Graphml, S2 Fig and S3 GraphML.

S2 Text: Description of the assembly and parameterisation of a model of the IAV life cycle in the context of a macrophage

S2 GraphML: GraphML file of the mEPN pathway representation of IAV life cycle in a cell with no host defences

S2 Fig: Construction and parameterisation of a representation of IAV life cycle in a cell with no host defences (A) Illustration of the modular structure of the IAV life cycle that facilitates its readability and allows for pathway expansion as new data becomes available or as a focus of interest grows. (B) When our virtual cell is challenged by a limited amount of virus (MOI 10 for 2 time blocks, 10 virions are represented by 10 input tokens, an accumulation of the structural components haemagglutinin (HA), neuraminidase (NA), matrix protein 1 (M1) and nonstructural protein 2 (NS2) both at the (C) mRNA and (D) protein level leading to (E)

viral progeny (~1E^4 virions/cell Virus Output). The model outputs are comparable to the levels of (F) mRNA and (G) protein accumulation seen in previously reported experimental *in vitro* infection.

S3 GraphML: GraphML file of the mEPN pathway representation of IAV life cycle in the context of a macrophage (IFNB-primed)

The challenges and rewards of pathway modelling: a biologist's perspective

Described above are the basic characteristics of the flow simulation algorithm and how this approach maps on to pathway diagrams assembled using the mEPN scheme. This interoperability between an advanced graphical notation scheme, and a powerful and rapid dynamic modelling platform opens up pathway modelling to a wider audience. However, modelling a pathway of any size is not a trivial undertaking – it takes skill, knowledge and dedication. From a biologist's perspective we would argue that the time and effort required to engage in this activity is rewarded. For most researchers a current pathway diagram describing their system of interest will not exist, and constructing it themselves can be an extremely useful exercise. It provides an opportunity for a biologist to formalise what they know in a form that can be communicated to their peers or co-workers. It also provides a platform where additional information can be added by others thereby offering a medium for debate and a route to establishing a common understanding. Indeed, one of the most valuable aspects to pathway modelling is the process of model construction itself, and the opportunity it provides to systematically collect and discuss available knowledge. By performing this act it exposes what is not known or as sometimes the case, why events as described could not possibly work the way they are reported to. In a teaching environment, students can be engaged in the activity. They gain the valuable experience in learning to mine the literature. They have to learn to be critical of the reliability of information and to judge how to represent information when incomplete or conflicting. How they go about

this task and what they produce in terms of a 'finished' diagram can be judged and marked in the same way as any written assignment.

Summary

The modelling framework described here is flexible enough to model all the basic network motifs found in all biological pathway types [44,45], as well as allowing curators to represent and capture pathway knowledge in great detail. It also supports the parameterization of models according to criteria that are readily accessible i.e., the relative amount of specific entities can often be experimentally determined or inferred, and tokens placed accordingly. Also, parameterisation of models such as introducing amplifications, reductions or delays to signal propagation can be made simply by marking such events on the models or by altering the structure of the model itself. We present a number of worked examples of pathway models drawn using the mEPN scheme on the '*Virtually Immune*' website (www.virtuallyimmune.org/) and BioLayout *Express*^{3D} as a means to model their dynamics. In these respects we believe that the computational framework presented here represents a major advance in modelling biological pathways. We also envisage that the tools and approaches described here will be useful to other groups. For instance those investigating single cell biology, where the response of individual cells in a population is heterogeneous and stochastic [46], or synthetic biology where the properties of an engineered biological pathway module could be tested prior to construction. We also envisage that the methodology will have application in the modelling of complex systems outside of biology.

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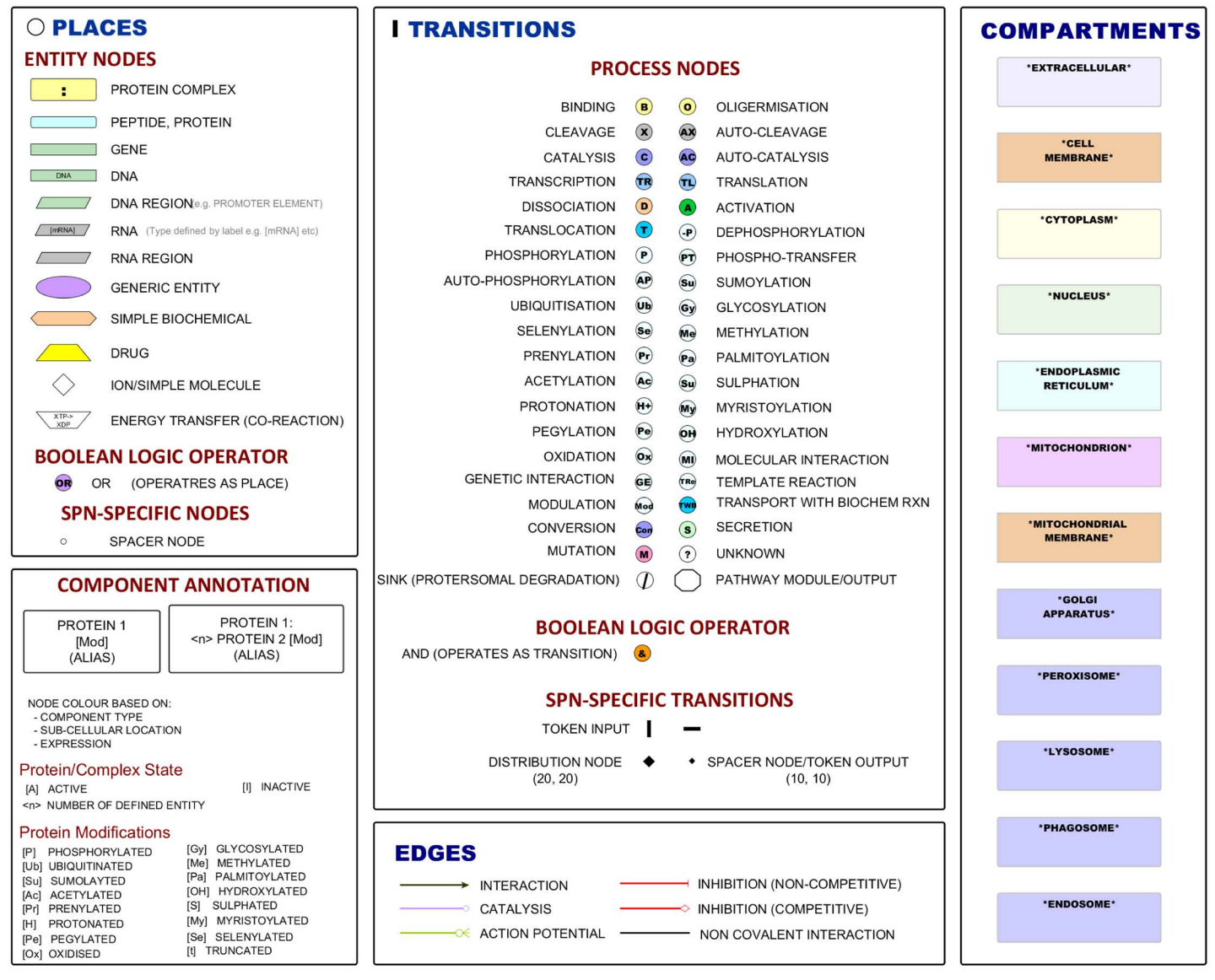
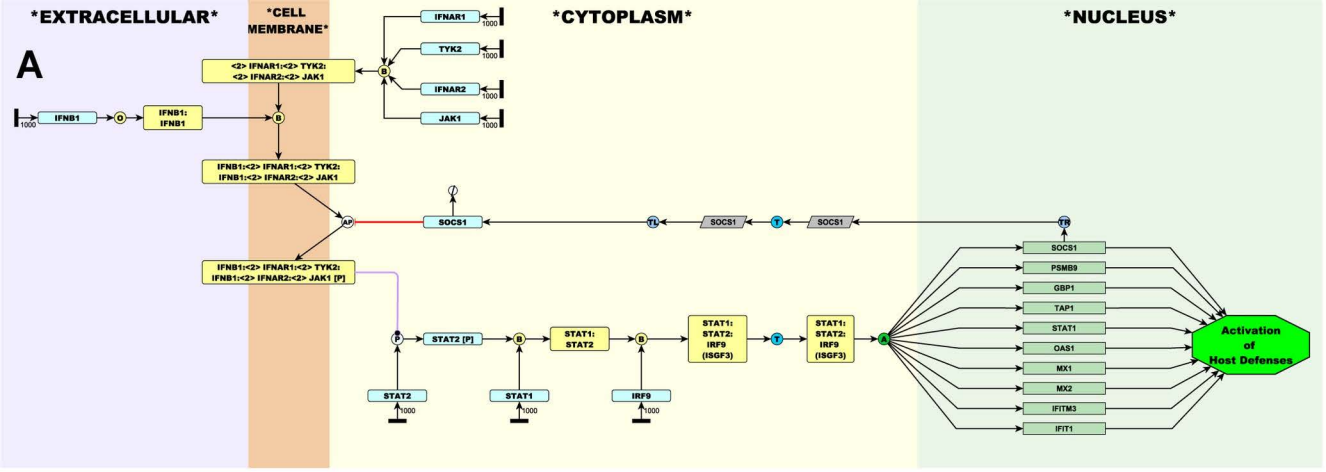
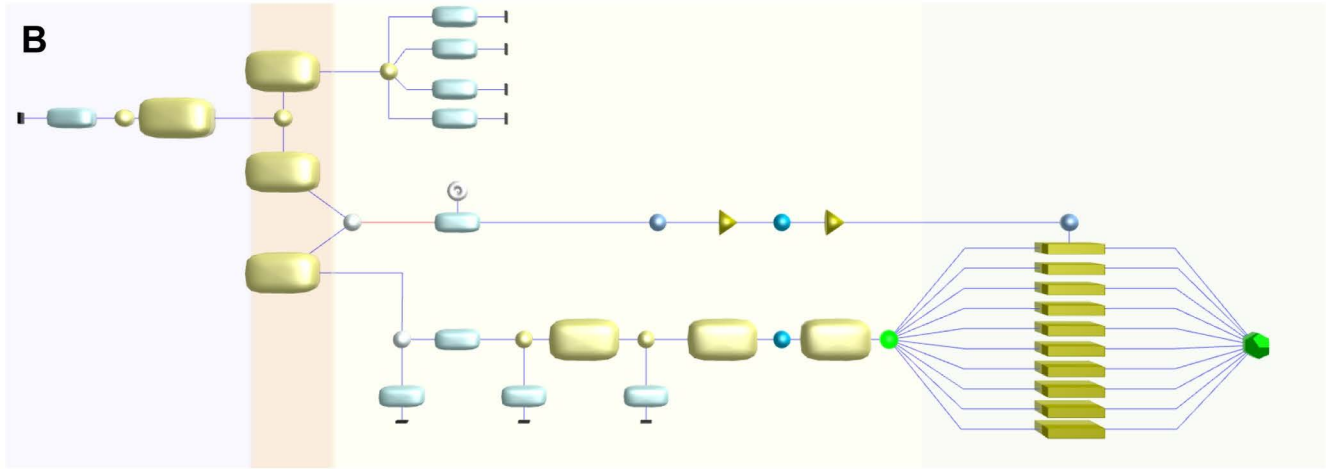
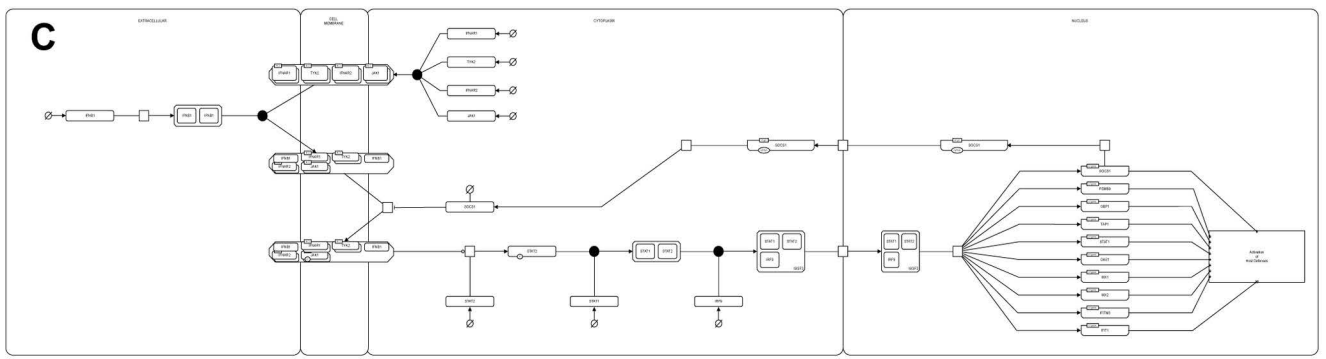
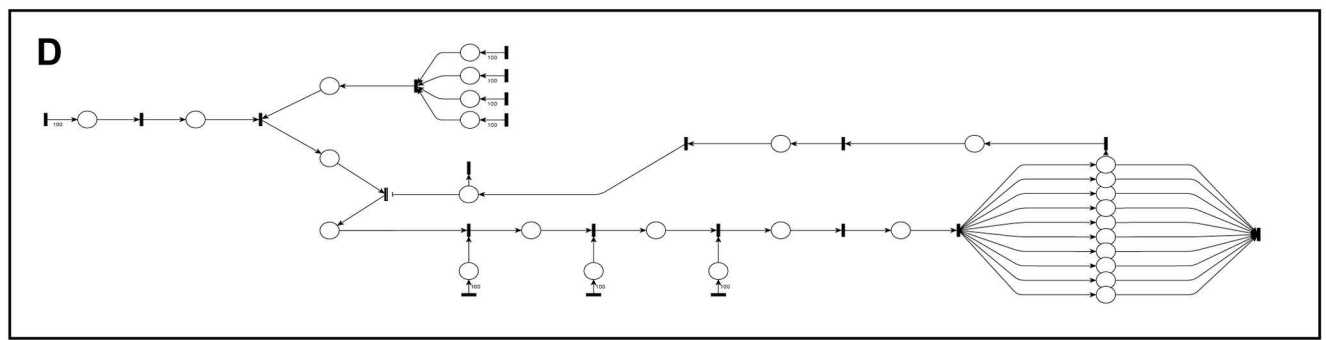

PLACES
ENTITY NODES
PROTEIN COMPLEX
PEPTIDE, PROTEIN
GENE
DNA
DNA REGION (e.g. PROMOTER ELEMENT)
[mRNA]
RNA (Type defined by label e.g. [mRNA] etc)
RNA REGION
GENERIC ENTITY
SIMPLE BIOCHEMICAL
DRUG
ION/SIMPLE MOLECULE
XTP-> XDP
ENERGY TRANSFER (CO-REACTION)
BOOLEAN LOGIC OPERATOR
OR
OR (OPERATRES AS PLACE)
SPN-SPECIFIC NODES
SPACER NODE
TRANSITIONS
PROCESS NODES
BINDING
CLEAVAGE
CATALYSIS
TRANSCRIPTION
DISSOCIATION
TRANSLOCATION
PHOSPHORYLATION
AUTO-PHOSPHORYLATION
UBIQUITISATION
SELENYLATION
PRENYLATION
ACETYLATION
PROTONATION
PEGYLATION
OXIDATION
GENETIC INTERACTION
MODULATION
CONVERSION
MUTATION
SINK (PROTERSOMAL DEGRADATION)
OLIGERMISATION
AUTO-CLEAVAGE
AUTO-CATALYSIS
TRANSLATION
ACTIVATION
DEPHOSPHORYLATION
PHOSPHO-TRANSFER
SUMOYLATION
GLYCOSYLATION
METHYLATION
PALMITOYLATION
SULPHATION
MYRISTOYLATION
HYDROXYLATION
MOLECULAR INTERACTION
TEMPLATE REACTION
TRANSPORT WITH BIOCHEM RXN
SECRETION
UNKNOWN
PATHWAY MODULE/OUTPUT
BOOLEAN LOGIC OPERATOR
AND (OPERATES AS TRANSITION)
SPN-SPECIFIC TRANSITIONS
TOKEN INPUT
DISTRIBUTION NODE (20, 20)
SPACER NODE/TOKEN OUTPUT (10, 10)
COMPARTMENTS
EXTRACELLULAR
CELL MEMBRANE
CYTOPLASM
NUCLEUS
ENDOPLASMIC RETICULUM
MITOCHONDRION
MITOCHONDRIAL MEMBRANE
GOLGI APPARATUS
PEROXISOME
LYSOSOME
PHAGOSOME
ENDOSOME
COMPONENT ANNOTATION
PROTEIN 1 [Mod] (ALIAS)
PROTEIN 1: <n> PROTEIN 2 [Mod] (ALIAS)
NODE COLOUR BASED ON:
- COMPONENT TYPE
- SUB-CELLULAR LOCATION
- EXPRESSION
Protein/Complex State
[A] ACTIVE
[I] INACTIVE
<n> NUMBER OF DEFINED ENTITY
Protein Modifications
[P] PHOSPHORYLATED
[Ub] UBIQUITINATED
[Su] SUMOLAYTED
[Ac] ACETYLATED
[Pr] PRENYLATED
[H] PROTONATED
[Pe] PEGYLATED
[Ox] OXIDISED
[Gy] GLYCOSYLATED
[Me] METHYLATED
[Pa] PALMITOYLATED
[OH] HYDROXYLATED
[S] SULPHATED
[My] MYRISTOYLATED
[Se] SELENYLATED
[t] TRUNCATED
EDGES
INTERACTION
CATALYSIS
ACTION POTENTIAL
INHIBITION (NON-COMPETITIVE)
INHIBITION (COMPETITIVE)
NON COVALENT INTERACTION


EXTRACELLULAR
CELL MEMBRANE
CYTOPLASM
NUCLEUS
A
IFNAR1
TYK2
IFNAR2
JAK1
<2> IFNAR1:<2> TYK2: <2> IFNAR2:<2> JAK1
IFNB1
IFNB1: IFNB1
IFNB1:<2> IFNAR1:<2> TYK2: IFNB1:<2> IFNAR2:<2> JAK1
SOCS1
SOCS1
SOCS1
IFNB1:<2> IFNAR1:<2> TYK2: IFNB1:<2> IFNAR2:<2> JAK1 [P]
STAT2 [P]
STAT1: STAT2
STAT1: STAT2: IRF9 (ISGF3)
STAT1: STAT2: IRF9 (ISGF3)
STAT2
STAT1
IRF9
SOCS1
PSMB9
GBP1
TAP1
STAT1
OAS1
MX1
MX2
IFITM3
IFIT1
Activation of Host Defenses


B


C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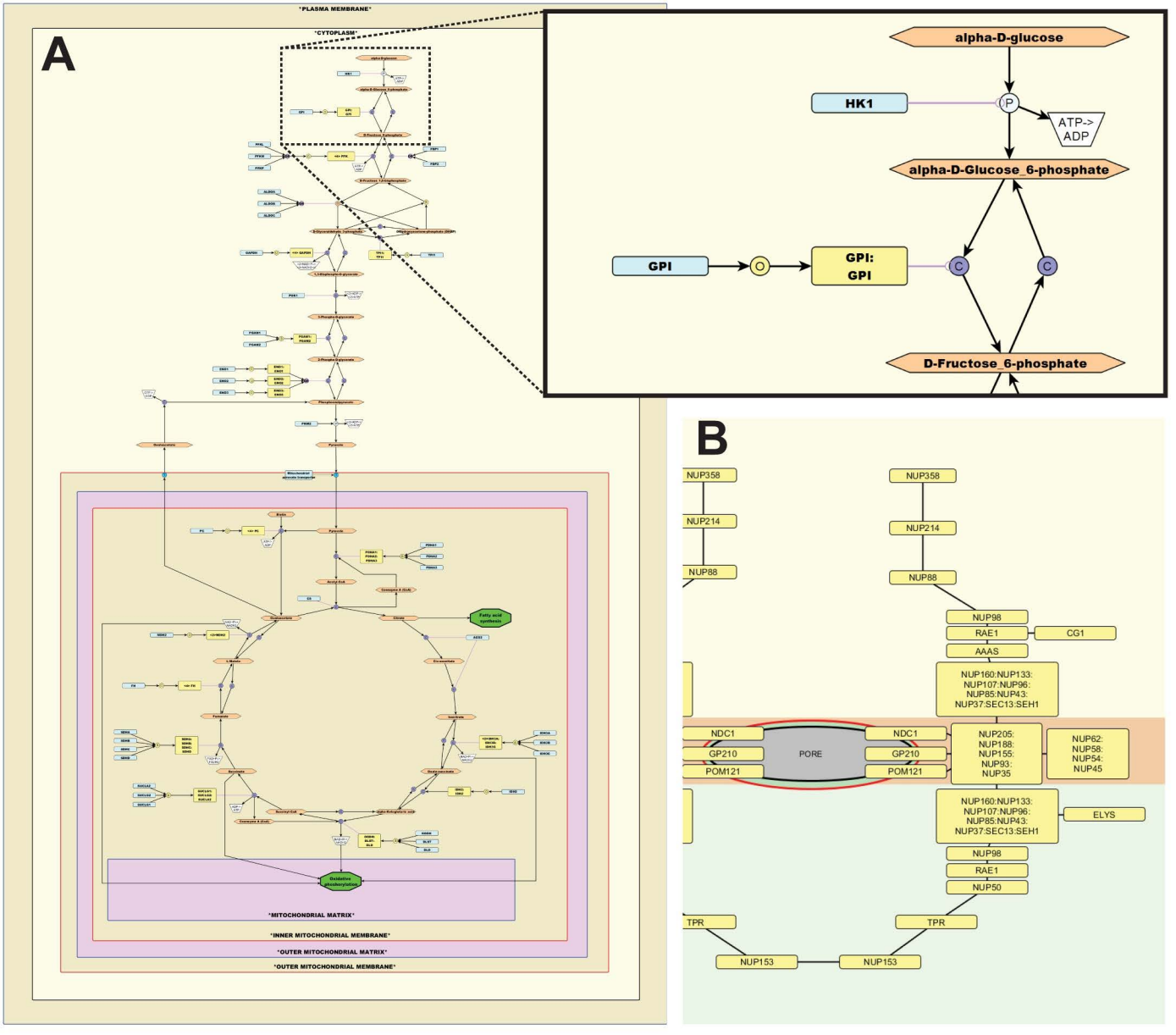
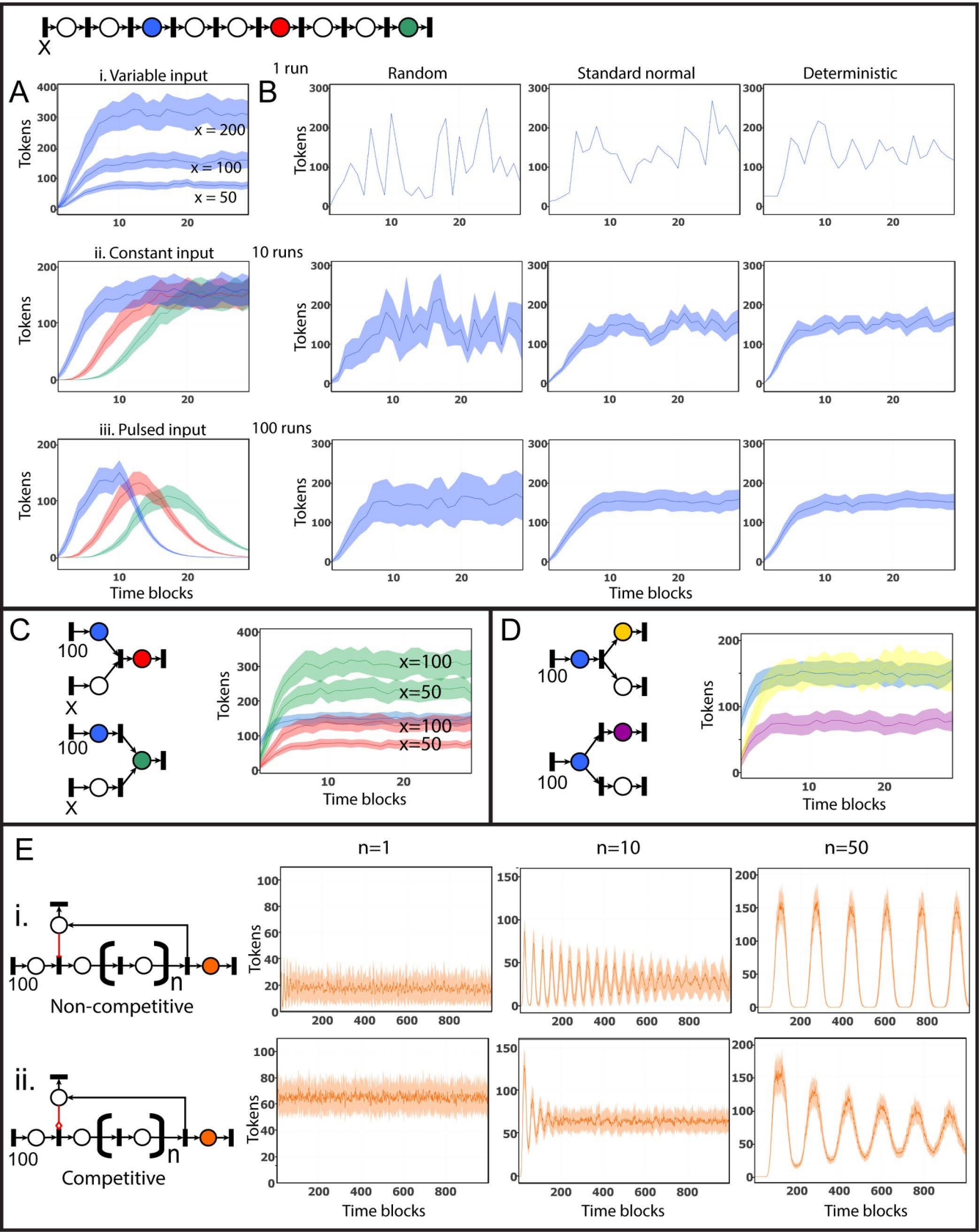
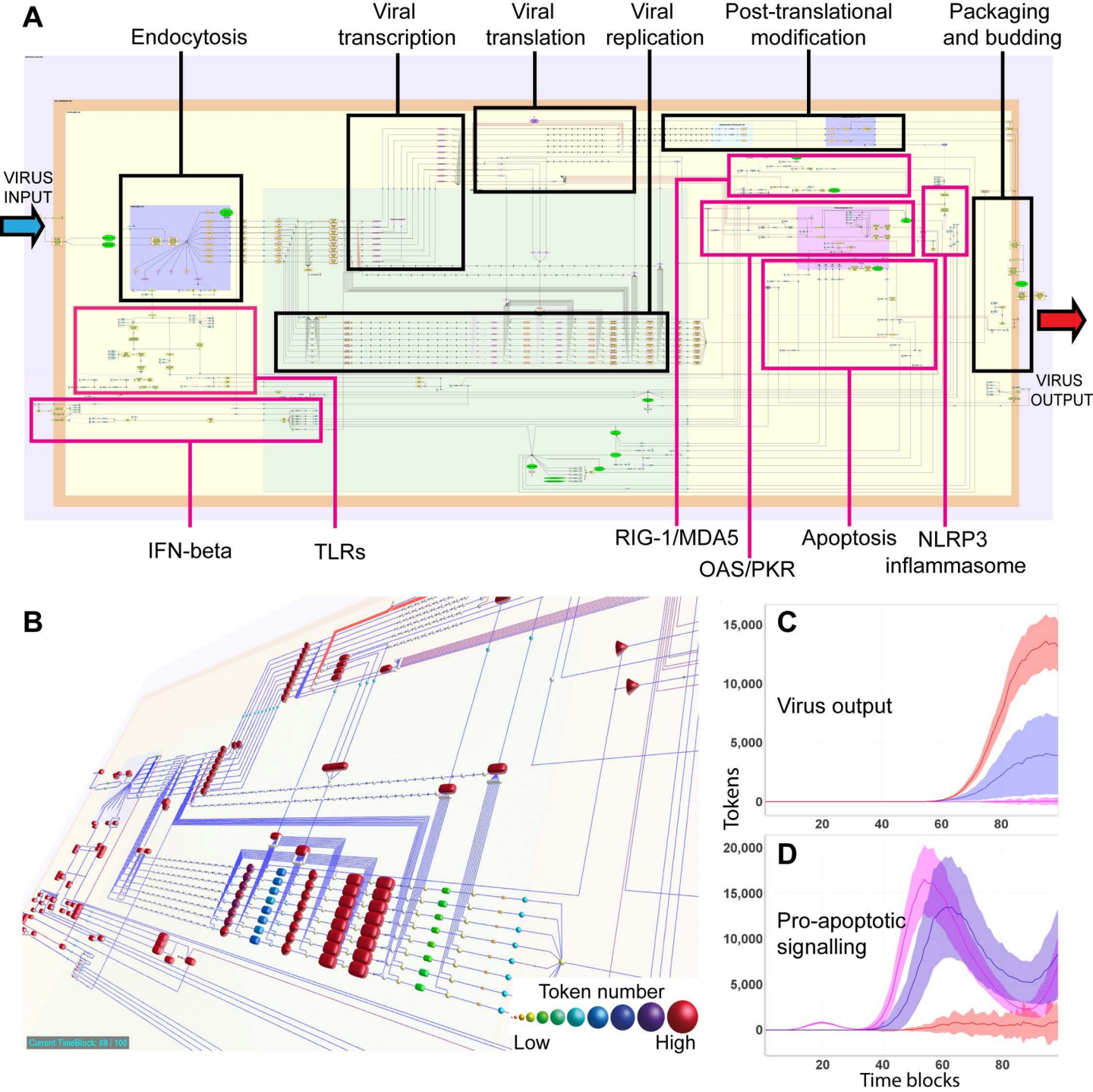

A
PLASMA MEMBRANE
CYTOPLASM
MITOCHONDRIAL MATRIX
INNER MITOCHONDRIAL MEMBRANE
OUTER MITOCHONDRIAL MATRIX
OUTER MITOCHONDRIAL MEMBRANE
alpha-D-glucose
HK1
P
ATP->
ADP
alpha-D-Glucose_6-phosphate
GPI
O
GPI:
GPI
C
C
D-Fructose_6-phosphate
B
NUP358
NUP214
NUP88
NUP98
RAE1
CG1
AAAS
NUP160:NUP133:
NUP107:NUP96:
NUP85:NUP43:
NUP37:SEC13:SEH1
NDC1
GP210
POM121
PORE
NDC1
GP210
POM121
NUP205:
NUP188:
NUP155:
NUP93:
NUP35
NUP62:
NUP58:
NUP54:
NUP45
NUP160:NUP133:
NUP107:NUP96:
NUP85:NUP43:
NUP37:SEC13:SEH1
ELYS
NUP98
RAE1
NUP50
TPR
TPR
NUP153
NUP153


x
A
i. Variable input
x = 200
x = 100
x = 50
Tokens
B
1 run
Random
Standard normal
Deterministic
ii. Constant input
10 runs
iii. Pulsed input
100 runs
Time blocks
C
100
x
D
E
n=1
n=10
n=50
i.
Non-competitive
ii.
Competitive
n


A
Endocytosis
Viral transcription
Viral translation
Viral replication
Post-translational modification
Packaging and budding
VIRUS INPUT
VIRUS OUTPUT
IFN-beta
TLRs
RIG-1/MDA5
OAS/PKR
Apoptosis
NLRP3 inflammasome
B
Token number
Low
High
Current TimeBlock: 68 / 100
C
Virus output
Tokens
15,000
10,000
5,000
0
20
40
60
80
D
Pro-apoptotic signalling
20,000
15,000
10,000
5,000
0
20
40
60
80
Time blocks